Registry No. H^+, 12408-02-5; NH, 13774-92-0; N^-, 18851-77-9; Sc^+, 14336-93-7; Ti^+, 14067-04-0; Y^+, 14782-34-4; Zr^+, 14701-19-0; Nb^+, 18587-63-8; La^+, 14175-57-6; Ta^+, 20561-66-4; V^+, 14782-33-3; Fe^+, 14067-02-8; Sc^+-NH, 115858-87-2; Ti^+-NH, 115858-88-3; Y^+-NH, 115858-89-4; Zr^+-NH, 115858-90-7; Nb^+-NH, 115858-91-8; La^+-NH, 115858-92-9; Ta^+-NH, 115858-93-0; V^+-NH, 115858-94-1; Fe^+-NH, 115858-95-2; V^+-C_3H_5, 115858-96-3; V-N, 24646-85-3; Cr^+, 14067-03-9; c-C_6H_6, 71-43-2; O_2, 7782-44-7; C_2H_4, 74-85-1; propene, 115-07-1; ammonia, 7664-41-7; *sec*-butylamine, 13952-84-6; *n*-propylamine, 107-10-8; pyridine, 110-86-1; diethylamine, 109-89-7; triethylamine, 121-44-8.

Surface-Enhanced Raman Study of the Effect of Electrode Potential and Solution pH upon the Interfacial Behavior of 4-Pyridinecarboxaldehyde

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Abstract: Surface-enhanced Raman scattering (SERS) spectra were obtained at a silver electrode in aqueous solutions at potentials from −0.2 to −0.6 V vs SCE and pH 1-7. The forms of 4-pyridinecarboxaldehyde that are adsorbed parallel those present in solution. In particular, the fraction of adsorbed species that is protonated at −0.2 V varies with pH in a manner almost identical with the fraction that is protonated in bulk solution. The protonated compound exists almost completely in the hydrated form (*gem*-diol) in the interface just as it does in solution. At a given pH, the fraction protonated on the surface increases as the potential is made more negative, a behavior attributed principally to enhanced adsorption of cationic species at negative potentials. In the presence of chloride, the fraction protonated decreases at the most negative potentials. This decrease is correlated with the desorption of chloride, suggesting that the pyridinium ion and chloride are adsorbed together. When the more strongly adsorbed bromide or iodide is used, the fraction protonated does not decrease at negative potentials.

A reactant molecule may exist in a variety of forms related to one another by acid-base reactions, complexation, tautomerization, etc. Frequently, one of these forms is more reactive than the others, causing the reaction to proceed via that particular species. A complete understanding of the reaction mechanism requires the identification of the reactive form, a task usually accomplished through a detailed evaluation of the reaction kinetics. When the reaction occurs at an interface (as in heterogeneous catalysis), it is highly desirable to have a very selective means of detecting adsorbed species while the reaction is occurring.

The reduction of aldehydes is an example of such a reaction. In aqueous solutions many aldehydes exist in two forms, the free aldehyde and the hydrate (*gem*-diol), with the relative concentrations being governed by a hydration equilibrium constant, K_h.

$$RCHO + H_2O = RCH(OH)_2$$

$$K_h = [RCH(OH)_2]/[RCHO]$$

In the electrochemical reduction of such aldehydes, it is the free aldehyde that is the active form. In order for the *gem*-diol to be reduced, it must first dehydrate to form free aldehyde, which in turn is reduced at the electrode surface. The dehydration reaction, which is often the rate-limiting step, can occur in solution near the electrode or, possibly, on the electrode surface.

The electrochemical reduction of 4-pyridinecarboxaldehyde has been the subject of intense study.[1-7] In acidic media over 90% of the aldehyde is hydrated,[1] but it can be rapidly and efficiently reduced to the alcohol, 4-pyridylcarbinol.[6] The dehydration reaction has been shown to be a crucial feature of the reduction mechanism under a variety of solution conditions.[1-3,5,7] Though the dehydration is normally considered to occur only in solution near the metal surface, a significant fraction may react on the surface, a possibility enhanced by the strong adsorption of pyridine and its derivatives.

In order to investigate the adsorption of the free and hydrated forms of 4-pyridinecarboxaldehyde, a selective and sensitive method is needed. Surface-enhanced Raman spectroscopy (SERS) is an attractive candidate. In fact, the original observations of SERS were obtained with pyridine at a silver surface.[8-10] As later investigations demonstrated, SERS is unusually powerful for in situ investigation of metal-solution interfaces. The sensitivity is excellent and the information content of the spectra is high, permitting resolution and identification of very similar surface species. In this paper we report the application of SERS to the characterization of the various forms of 4-pyridinecarboxaldehyde adsorbed at silver surfaces as a function of solution pH and electrode potential. As will be seen, systematic investigation of the effects of these variables proved to be crucial in the detection of the adsorbed reactive free aldehyde, a species that was not

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present at the pH and potential used in the only previous SERS investigation of 4-pyridinecarboxaldehyde, that of Bunding and Bell.[11]

Experimental Section

4-Pyridinecarboxaldehyde (Aldrich Chemical Co.) was distilled at reduced pressure and stored under a nitrogen atmosphere prior to use. Water was triply distilled, and all other reagents were analytical reagent grade.

Acetate buffers were used for pH values between 3.5 and 5.5 while phosphate buffers were used for pH 5.5–7. The pH of solutions below pH 3.5 was adjusted with HCl. All solutions contained 0.10 M KCl (or 0.10 M KBr or KI in certain experiments as specified), and the ionic strength of the buffers was adjusted to 0.90 M by addition of potassium nitrate.

The SERS cell was built following the design of Brandt.[12] The polycrystalline silver electrode was a disk (3-mm radius) prepared by press-fitting a cylinder of silver into one end of a 9.5-mm-diameter Teflon rod through which a 6-mm-diameter concentric hole had been drilled. Electrical contact was made via a copper wire soldered to the silver. Prior to the experiment, the silver electrode was polished with 5-, 0.3-, and 0.05-μm alumina. After polishing, the electrode surface was rinsed with copious amounts of triply distilled water followed by sonication in triply distilled water. The SERS cell was then assembled and filled with an analyte solution, which had been previously purged with nitrogen. The silver electrode was then subjected to an oxidation–reduction cycle (ORC), viz., oxidation at 0.20 V for 10 s followed by reduction of the generated silver chloride at −0.30 V until the current nearly ceased. When KBr was used in the electrolyte, the potentials were +0.20 and −0.60 V, and with KI −0.20 and −0.80 V were selected. About 100 mC/cm^2 was passed in the oxidation and reduction steps. For convenience, all spectra reported were obtained after an ORC with the solute present. When the solute was added after the ORC, very similar spectra were recorded.

The spectra were taken with the 488.0-nm line of a Spectra Physics Model 164-00 argon ion laser with an incident power of 100 mW at the electrode surface. The laser light was focused to a line image at the electrode by a cylindrical lens. The scattered light was focused onto the entrance slit (2.0-cm^{-1} resolution) of a Spex Model 1401 double monochromator, and detection was by photon counting with an RCA Model C31034-02 photomultiplier tube. Data collection was performed with a microcomputer system. The intensity of the SERS spectra varied considerably with changes in conditions. All spectra have been plotted with an arbitrary scale for the ordinate. Sharp lines at 223, 738, 1010, 1055, and 1578 cm^{-1} that appear in various spectra are from scattered argon ion emission.

The electrode potential was controlled by a Princeton Applied Research EG&G (PAR) Model 173 potentiostat/galvanostat and is referenced to a saturated calomel electrode. A PAR Model 276 current-to-voltage converter allowed monitoring of current during the ORC and SERS experiments. Positive-feedback *iR* compensation was employed though the ohmic losses were low in the SERS experiments due to the inherently low currents.

Results and Discussion

Detection of Adsorbed Free Aldehyde. SERS spectra of 0.050 M 4-pyridinecarboxaldehyde in 0.10 M KCl at various electrode potentials are shown in Figure 1. As the electrode potential is made more negative, the spectrum undergoes dramatic changes. Features at 1070, 1250, 1325, 1420, 1470, 1490, 1535, and 1560 cm^{-1} appear and become more intense as the potential is made more negative. Additionally, a small feature at 1640 cm^{-1} appears at about −0.40 V, and this band also increases in intensity at more negative potentials. As will be shown later, the band at 1640 cm^{-1} is due to the protonated pyridine and will be very valuable in characterizing the adsorbed species.

Significantly, at −0.20 V there is a small but easily detectable band corresponding to the carbonyl stretching frequency at 1710 cm^{-1}. It is also potential-dependent, becoming weaker and finally disappearing altogether at about −0.5 V. This confirms the result of Bunding and Bell[11] who reported no carbonyl band because they obtained their spectra at a single potential, −0.60 V.

Thus, the reducible form of 4-pyridinecarboxaldehyde, the free aldehyde, is adsorbed at the silver electrode as long as the potential

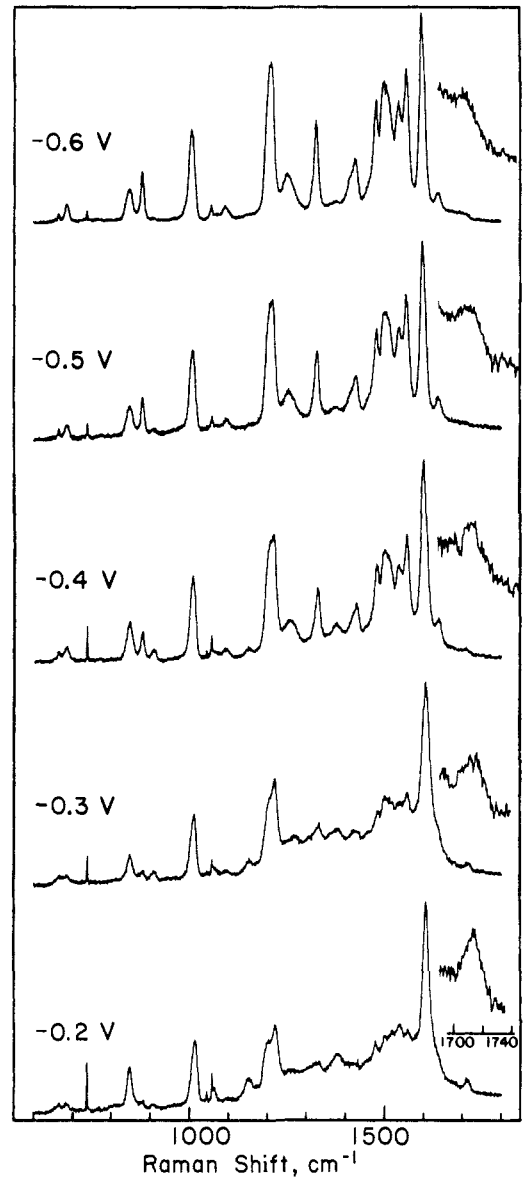

Figure 1. SERS spectra of 0.050 M 4-pyridinecarboxaldehyde in aqueous 0.10 M KCl using a silver electrode. Potentials are vs SCE. Insets show expanded view of carbonyl band near 1710 cm^{-1}.

is not too negative. At these less negative potentials (−0.2 to −0.4 V), 4-pyridinecarboxaldehyde does not completely hydrate upon adsorption as was suggested by Bunding and Bell.

Allen and Van Duyne[13] have considered orientational effects on band intensities and have concluded that the relative intensities should be given by eq 1, where the intensity of the carbonyl band

$$(I_{1710}/I_{1000})_{SERS} = (I_{1710}/I_{1000})_{NRS}(\cos^2\theta) \qquad (1)$$

is expressed relative to the intense symmetrical ring-breathing mode near 1000 cm^{-1}, in both the SERS and normal Raman solution spectra (NRS). θ is the angle of the carbon–oxygen bond in the adsorbed species with respect to the surface normal. For vertical orientation of 4-pyridinecarboxaldehyde (adsorption via the ring nitrogen atom), θ will be 60°. Analysis of the SERS data at −0.20 V and NRS (0.10 M KCl) gave θ = 57°, suggesting a vertical orientation at this potential. However, some process leads to the diminution of the carbonyl band intensity as the potential is made negative.

Possibly the free aldehyde is reduced at the electrode, causing the disappearance of the band at 1710 cm^{-1}. However, cyclic voltammetry of 0.010 M 4-pyridinecarboxaldehyde at a silver electrode in 0.10 M KCl reveals virtually no faradaic current at

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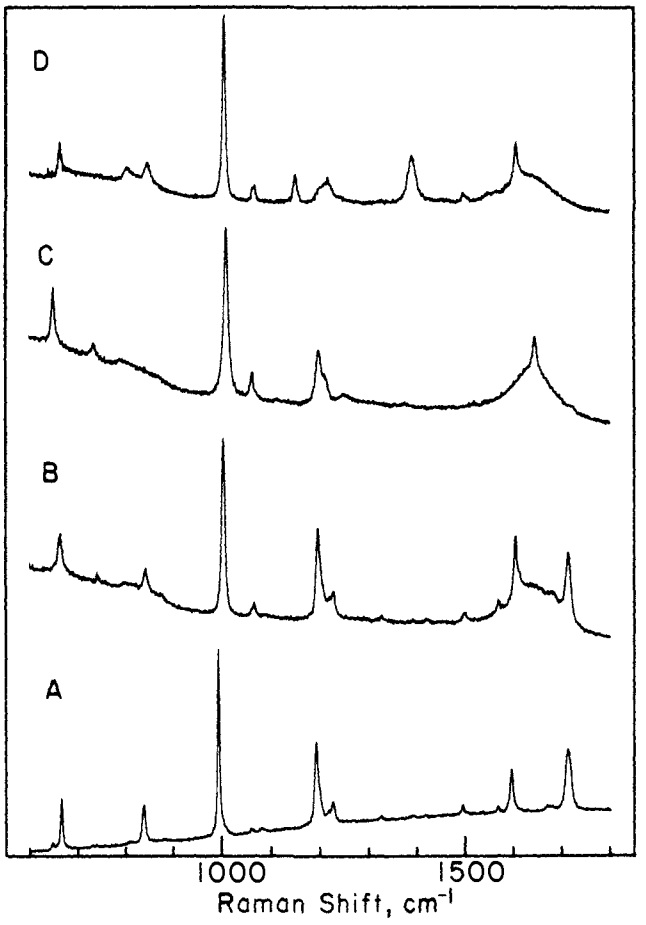


Figure 2. Normal Raman spectra of 4-pyridinecarboxaldehyde: (A) pure liquid; (B) 0.10 M aqueous solution; (C) 0.10 M in 0.50 M HCl; (D) 0.10 M in 0.50 M NaOH.

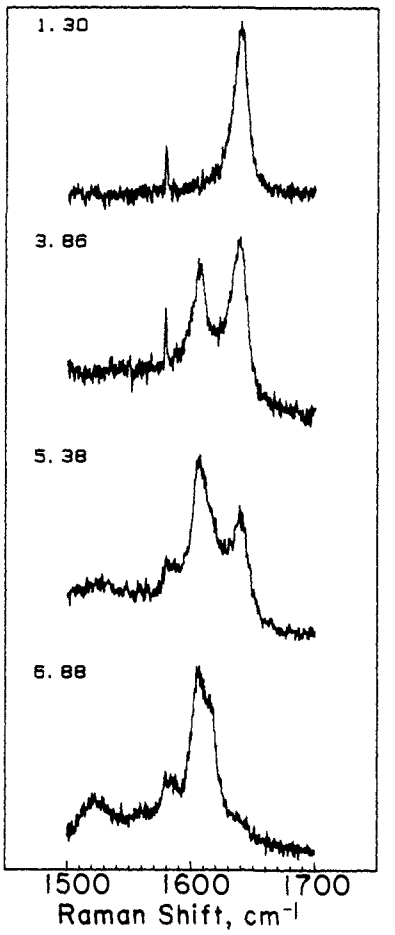
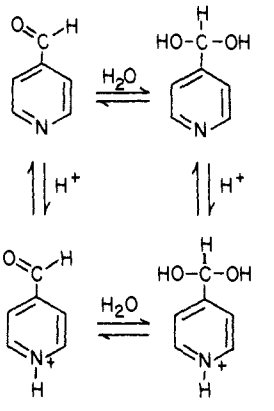


Figure 3. SERS spectra of 0.050 M 4-pyridinecarboxaldehyde in various buffers of the pH indicated. All spectra were obtained at −0.20 V vs SCE.

potentials as negative as −0.8 V, making it highly unlikely that the adsorbed free aldehyde is being reduced at even the most negative potential used in the SERS studies. As suggested by Bunding and Bell,[11] hydration of the surface species may be responsible for the diminution of the carbonyl Raman band. To explore this possibility, it will be helpful to examine the acid–base and hydration equilibria of 4-pyridinecarboxaldehyde in aqueous solutions.

Spectroscopic Behavior of 4-Pyridinecarboxaldehyde as a Function of Solution pH. It has long been known that the extent of hydration of 4-pyridinecarboxaldehyde depends upon the solution pH. At pH <3, the pyridine nitrogen is protonated, which promotes hydration of the aldehyde group, giving about 95% hydrate according to the equilibrium constants of Laviron[1] (cf. Scheme I). By contrast, for 5.5 < pH < 11, the pyridyl ring is not protonated and only about half exists as the hydrate. These equilibria have been investigated by a number of techniques[1,14-21] and are easily detected by Raman spectroscopy as well. Figure 2 shows the normal Raman spectra of neat 4-pyridinecarboxaldehyde and 0.10 M solutions in water, 0.50 M HCl, and 0.50 M NaOH. Many differences are noted among the four spectra, but two specific changes are most informative.

Scheme I



First, the carbonyl band at about 1710 cm^{-1} is prominent in the neat liquid and in the aqueous solution where about half of the compound exists in the free aldehyde form. However, it is not detectable in the acidic medium where about 95% is hydrated. (It is also absent in strong base where the anion of the *gem*-diol is favored.[1,5])

Second, a very useful feature is the behavior of the band near 1600 cm^{-1} seen in the spectrum of the neat liquid and the aqueous solution. This is a ring mode, which shifts markedly upon protonation of the pyridyl ring. In the spectrum of the acidic solution (in which 4-pyridinecarboxaldehyde is completely protonated), the band at 1600 cm^{-1} is absent and has been replaced by a new feature at about 1640 cm^{-1}. This shift to higher frequencies upon protonation of the ring nitrogen permits the monitoring of the

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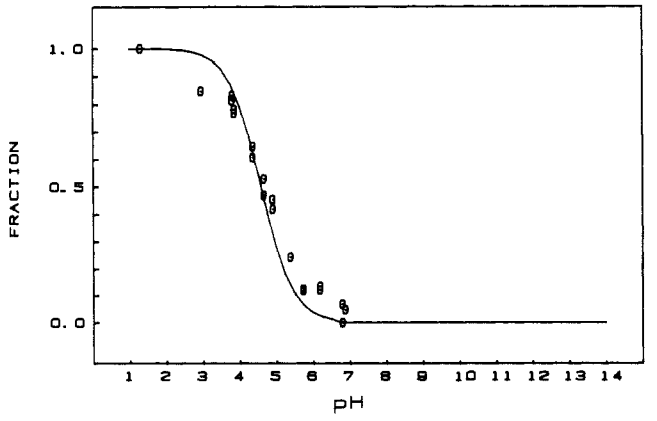

Figure 4. Surface mole fraction of protonated forms of 4-pyridinecarboxaldehyde as obtained from SERS spectra at various pH. The curve is the mole fraction of protonated forms present in solution calculated from solution-phase equilibrium constants.

extent of protonation using NRS and SERS. It is a common behavior, which has been reported in the Raman spectra of a variety of monosubstituted pyridines[22-25] as well as pyridine itself.[9,25]

With this assignment of bands due to neutral and protonated forms of 4-pyridinecarboxaldehyde in hand, we can examine the effect of solution pH on the SERS spectra (Figure 3). These spectra were obtained at −0.20 V and, as the solution pH was decreased, the relative intensity of the band at 1640 cm^{-1} (protonated) increased while that at 1600 cm^{-1} (neutral) waned. In addition, as the amount of protonated species on the surface increased, the weak band for carbonyl at about 1710 cm^{-1} completely disappeared. This is consistent with the fact that the protonated form is as much as 95% hydrated in the bulk of solution. Apparently it is mostly hydrated on the surface as well.

To estimate the relative populations of the protonated and neutral forms on the surface, the band heights were measured and an intensity ratio was calculated, $I_{1640}/(I_{1600} + I_{1640})$. If the scattering cross sections are the same, this intensity ratio will equal the mole fraction of the pyridine species on the surface that is protonated. Such an assumption is reasonable because the bands are thought to arise from the same vibrational mode though it must be admitted that the cross sections might differ due to other factors, such as differences in electrode-adsorbate distance for the neutral and protonated forms. Presumably, both the free and hydrated forms contribute to the bands for the neutral (1600 cm^{-1}) and protonated (1640 cm^{-1}) species.

The intensity ratios from SERS spectra obtained at −0.20 V in a variety of buffers are plotted in Figure 4 along with the fraction of protonated 4-pyridinecarboxaldehyde (free and hydrate) present in the solution, as calculated from known solution equilibrium constants.[1] As can be seen from Figure 4, the relative population of the protonated 4-pyridinecarboxaldehyde species at the silver surface at −0.20 V vs SCE is approximately equal to the relative population in the bulk solution; i.e., at this potential the protonated and neutral forms have the same adsorption coefficient. This is not the case at other potentials, however, as will be demonstrated below.

Examination of the Potential Dependence of the SERS Behavior of 4-Pyridinecarboxaldehyde. We will now focus on the bands at 1600 and 1640 cm^{-1} to determine the effect of electrode potential on the relative surface populations of neutral and protonated forms. A pH 6.8 buffer was selected with which no protonated form could be detected (intensity ratio 0.0) at −0.20 V. Spectra are presented at Figure 5 where it may be seen that the relative intensity of the band at 1640 cm^{-1} increases as the electrode potential is changed to more negative values.

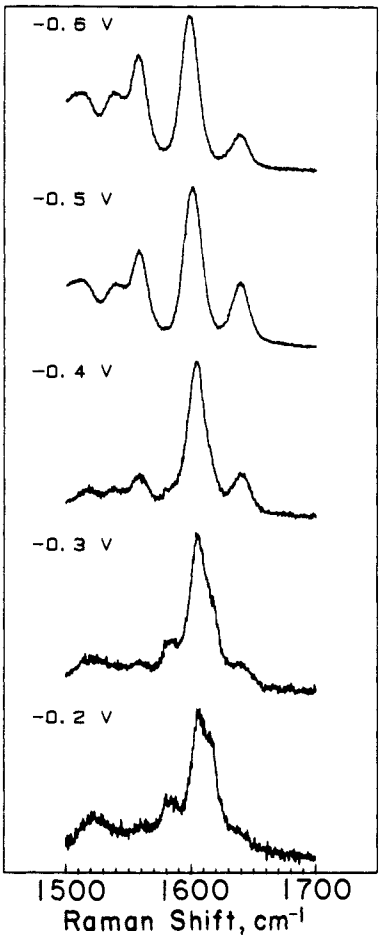

Figure 5. SERS spectra of 0.050 M 4-pyridinecarboxaldehyde in pH 6.88 buffer as a function of electrode potential, V vs SCE.

Table I. Surface Mole Fraction of 4-Pyridinecarboxaldehyde Which Is Protonated[a]

	mole fraction protonated			
E, V vs SCE	0.10 M KI	0.10 M KBr[c]	0.10 M KCl[c]	pH[b]
−0.20		0.10	0.00	6.8
−0.30		0.17	0.04	6.0
−0.40		0.24	0.16	5.3
−0.50	0.40	0.30	0.27	4.9
−0.60	0.40	0.43	0.15	5.3

[a] Mole fraction was calculated from intensity ratio of SERS peaks for protonated (1640 cm^{-1}) and neutral (1600 cm^{-1}) species given by $I_{1640}/(I_{1600} + I_{1640})$. [b] The solution pH giving the same mole fraction of protonated species in solution as was seen for the surface species in the chloride-containing solutions. [c] Solution also contained the pH 6.88 buffer.

In the SERS spectra, the effect of changing the electrode potential to more negative values is the same as the effect of lowering the solution pH. Table I lists the surface mole fraction of protonated species obtained from the SERS spectra of Figure 5 along with the pH which would produce the same mole fraction in solution. From Table I it can be seen that changing the electrode potential from −0.20 to −0.50 V (chloride-containing medium) has the same effect upon the spectrum as lowering the pH from 6.88, the bulk value, to about 5.

The effects of pH and electrode potential upon the surface coverages of the two forms of a conjugate acid–base pair have been considered earlier,[26-29] usually with respect to capacitance

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data obtained with mercury electrodes. In a study of the adsorption of pyridine and pyridinium ion, Galus et al.[26] found an effect precisely analogous to that seen in the SERS spectra of 4-pyridinecarboxaldehyde; viz., at a given pH the relative surface concentration of pyridinium increased as the potential was made negative. The effect was smaller, however, as the change was equivalent to only about half unit decrease in solution pH.

A contributor to this effect is that the concentration of hydrogen ions in the double layer will be affected by the potential there compared to the bulk solution. If we suppose that the potential at the outer Helmholtz plane is the appropriate potential, it will decrease as the potential is made negative, resulting in an increase in the concentration of hydrogen ions in the double layer. However, at the high ionic strength employed in the present work, the double-layer potential will be small,[28] and its change with potential is likely to cause only a minor decrease in surface pH, much less than the nearly two unit change implied by the data of Table I.

A more important factor is the potential dependence of the adsorption coefficients of the protonated and neutral pyridine species.[27,28] As was pointed out in the discussion of Figure 4, the adsorption coefficients are quite similar at −0.20 V, but, as the potential is made more negative, adsorption of the cationic form will be enhanced, in accord with the data of Figure 5.

Thus, the SERS spectra provide a direct means of monitoring the relative concentrations of protonated and neutral forms on the surface and of investigating the effects of solution pH and potential. Gratifyingly, the results are in consonance with the trends seen in studies of related compounds based upon electrode capacitance. An important advantage of the SERS measurements is their molecular specificity. For example, the spectra of Figure 5 show that the surface mole fraction of protonated 4-pyridinecarboxaldehyde increases at negative potentials. The question arises as to whether this protonated species is the free aldehyde or the hydrate. Examination of the weak carbonyl band at 1710 cm^{-1} reveals that it again diminishes and finally disappears near −0.5 V. Thus, the protonated species on the surface is predominantly hydrated, just as it is in solution. It is intriguing that the relative surface population of the reducible form of 4-pyridinecarboxaldehyde, the free aldehyde, actually decreases as the potential is moved toward values where the reduction reaction can proceed.

Adsorption of Protonated 4-Pyridinecarboxaldehyde. One feature of the data in Figure 5 has not been mentioned. The relative intensity of the band due to the protonated species increases from −0.20 to −0.50 V, but it decreases again on going to −0.60 V. This observation prompts a more thorough analysis of the adsorption of the protonated pyridine derivative.

Though the phenomenon was first discovered with neutral pyridine, SERS spectra of protonated pyridine have been reported previously.[30-34] It has been concluded that the cation is adsorbed as an ion pair with chloride,[31-34] a conclusion supported by the observation that bands due to pyridinium ions diminish as the potential is made negative and chloride is desorbed from the electrode. Very recently, similar observations have been made for the protonated groups in poly(2-vinylpyridine).[35] It is probable that the protonated forms of 4-pyridinecarboxaldehyde also adsorb as an ion pair with chloride, and the decrease in the relative intensity of the band at 1640 cm^{-1} on going from −0.50 to −0.60 V is due to the desorption of chloride. To test this suggestion, the band at 240 cm^{-1}, assigned to adsorbed chloride,[33,34,36-38] was monitored as a function of electrode potential (Figure 6). The band is clearly present at all potentials from −0.20 to −0.50 V, but it diminishes considerably at −0.60 V and is almost absent at −0.70 V, providing direct detection of the desorption of chloride.

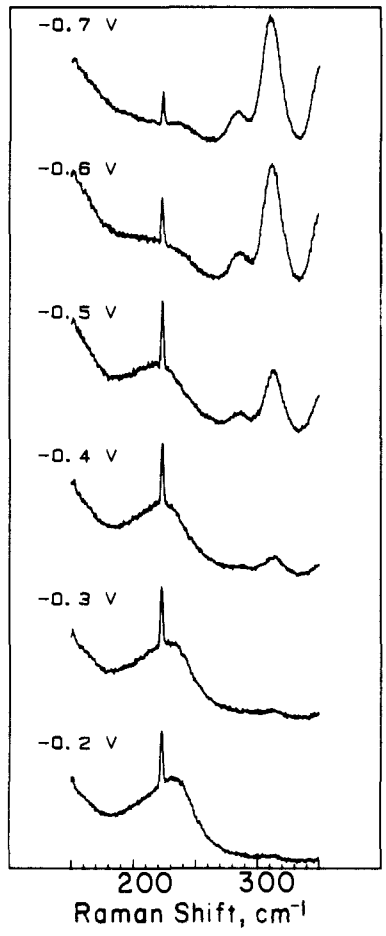

Figure 6. SERS spectra of the Ag–Cl stretching mode (240 cm^{-1}) for aqueous 0.10 M KCl solution containing 0.050 M 4-pyridinecarboxaldehyde as a function of electrode potential, V vs SCE.

When the surface mole fraction of protonated species as a function of potential was obtained in the presence of KBr, the mole fraction was observed to increase smoothly from −0.20 to −0.60 V (Table I), reflecting the enhanced adsorption of the cationic forms at negative potentials. No decrease in the surface mole fraction of protonated species is seen at potentials as negative as −0.60 V, in agreement with the fact that bromide is more strongly adsorbed than chloride and a more negative potential is required to cause desorption.[34,38] In the presence of iodide, potentials more positive than about −0.40 V cannot be used due to oxidation of the silver surface, but a large and constant surface mole fraction of protonated species was found at −0.50 and −0.60 V (Table I), consistent with the strong adsorption of iodide and the fact that it is not desorbed unless the potential is more negative than −0.70 V.[34,38]

Conclusion

Both the reactive form of 4-pyridinecarboxaldehyde (the free aldehyde) and the hydrate are adsorbed at the silver–aqueous solution interface. At low pH and/or negative potentials, the relative amount of adsorbed free aldehyde decreases. This is associated with the increase in the relative surface population of the protonated aldehyde; i.e., the protonated species on the surface exists almost entirely as the hydrate, just as it does in solution. The increase in the relative concentration of protonated 4-pyridinecarboxaldehyde on the surface as the potential is made

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more negative is caused by the enhanced adsorption of the cationic forms at negative potentials. In accord with earlier studies of similar compounds, the protonated forms appear to adsorb in association with halide ions.

This study shows that near pH 7, both the neutral free aldehyde (the reducible form) and the neutral hydrate are adsorbed. However, for $1 < pH < 4$, most of the adsorbed aldehyde is protonated (Figure 3), and, consequently, very little free aldehyde is present. Nevertheless, the reduction reaction is not impeded. In separate experiments, we found that diffusion-controlled reduction of the free aldehyde was observed by chronoamperometry at pH 3.86 and 6.88 at both silver and mercury electrodes. We conclude that either the reactant need not adsorb before the reduction occurs or, if it must adsorb, this step is not rate-limiting.

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Registry No. 4-Pyridinecarboxaldehyde, 872-85-5; silver, 7440-22-4; chloride, 16887-00-6; bromide, 24959-67-9; iodide, 20461-54-5; protonated 4-pyridinecarboxaldehyde hydrate, 105868-55-1.

Resonance Raman Studies of Hydrogenase-Catalyzed Reduction of Cytochrome c_3 by Hydrogen. Evidence for Heme–Heme Interactions

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Abstract: We observed splittings of Raman bands for a multi-heme protein for the first time. In the resonance Raman studies of hydrogenase-catalyzed reduction of a tetraheme protein cytochrome c_3 (Cyt-c_3) with hydrogen, certain Raman bands show clear splittings in the intermediate redox states obtained under controlled pressure of hydrogen, while apparently nonstructured bands were observed in the fully oxidized or fully reduced states. This suggests either nonequivalence in the environments of the four hemes or an exciton-like splitting of vibrational modes. Since various C-type cytochromes at neutral pH, which have different amino acid compositions but the same axial ligands, do not give a wide variety of frequencies for a given mode and, furthermore, a bandwidth of the Soret band was much narrower with Cyt-c_3 than with various single-heme cytochromes c, it is more likely to assume that the four hemes in the protein interact directly with each other, giving rise to exciton-like splitting. Observation of well-defined Raman lines in the partially reduced states at the characteristic frequencies for the reduced or the oxidized form indicates that the electron-exchange rate, either intramolecular within the four hemes or intermolecular between different Cyt-c_3 molecules, is much slower than the time scale of the RR scattering process while the recent NMR studies indicate that the intramolecular electron-exchange rates are faster than 10^9 s^{-1}. We have attempted to estimate the relative importance of the dispersion, induction, orientational, and repulsive interactions for understanding the splitting of the Raman bands. The dipole–dipole coupling mechanism can be ruled out while the dispersion-type interactions may contribute predominantly to it.

Cytochromes c_3 (Cyt-c_3), which are found in the strictly anaerobic sulfate reducing bacteria belonging to the genus *Desulfovibrio*, constitute a class of proteins containing four C-type hemes bound covalently to a single polypeptide with $M_r = 13\,000$. All four heme irons in each molecule are coordinated by two histidine residues, adopting the hexacoordinated low-spin form in both the oxidized and reduced states of the protein. Physiologically Cyt-c_3 acts as an electron carrier of *Desulfovibrio* hydrogenase (hydrogen:ferricytochrome c_3 oxidoreductase, EC 1.12.2.1)[2] and plays a key role in the complex sulfate reduction metabolism of *Desulfovibrio*. This protein is very interesting since the anhydrous solid film of its reduced form on a quartz plate exhibits almost metallic conductivity in magnitude and there is a 10^{13}-fold difference in the electrical resistivity between the oxidized and reduced forms.[3a-c]

The physicochemical properties of the five varieties of Cyt-c_3 from different species in the genus *Desulfovibrio* have been investigated by several groups using optical absorption,[2,4,5] nuclear magnetic resonance,[6,7] electron spin resonance,[8,9] Mössbauer,[10] electrical conductivity,[3] ionization potentials,[11] and electrochemical[12,13] techniques (see ref 13 for a review). One of the peculiar features of the Cyt-c_3 is its very negative redox potential and it can be reduced by hydrogen in the presence of hydrogenase. The electrochemical,[4] NMR,[6,7] and ESR[9] studies suggest that the Cyt-c_3 accepts four electrons in a stepwise manner and the different redox states are characterized by four separate but closely spaced negative redox potentials. Recent X-ray diffraction studies[14,15] on the two species of Cyt-c_3 show that the relative disposition of

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